SPOTLIGHT feature

Food & Beverage Analysis

Five Key Practices for Pesticide Analysis in Challenging Food Matrices by GC/MS

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Global food demand is rising, and so is the use of pesticides, with over a thousand different chemicals on the market [1]. However, pesticide residues can pose risks to the global food supply chain, the environment, and the consumer when proper agricultural practices are not followed.

Concerns about trace level pollutants in food are driving the demand for more rapid and reliable ways to identify and quantify chemical residues. One measure of food safety is the maximum residue limit (MRL), which is the highest level of pesticide residue allowed to remain in or on the treated food commodity. In this article we highlight five key ways to ensure successful pesticide analysis using gas chromatography / triple quadrupole mass spectrometry.

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Concerns about trace level pollutants in food are driving the demand for more rapid and reliable ways to identify and quantify chemical residues. One measure of food safety is the maximum residue limit (MRL), which is the highest level of pesticide residue allowed to remain in or on the treated food commodity. For example, in the US, MRLs are known as tolerances [2].

MRLs may vary over a broad concentration range depending on the type of pesticide and the food product treated. For example, the US Environmental Protection Agency (EPA) has established tolerances for 68 pesticides in spinach that vary from 10 parts per billion (ppb) for fludioxonil to 60,000 ppb for boscalid [3].

This range of limits presents a challenge for gas chromatography/mass spectrometry (GC/MS) analysis, requiring both high sensitivity and the ability to calibrate over a wide dynamic range. Five key components can ensure successful pesticide analysis:

- Effective sample extraction and matrix cleanup
- Evaluation of the matrix in full scan data acquisition mode
- Midcolumn backflushing
- A leak-free triple quadrupole GC/MS system (GC/TQ)
- Proper inlet and liner selection

Here, we describe how these five components enabled the GC/MS/MS analysis of over 200 pesticides in three challenging matrices: a high chlorophyll fresh matrix spinach, a complex dry matrix cayenne pepper, and an oily dry matrix walnut. Two separate GC/TQ models (*Figures 1A and 1B*) were used and configured to achieve the best performance over a wide calibration range. GC/MS/MS is extremely selective, sensitive, linear, and robust technic used for quantitating pesticides in various matrices. It is often used in combination with liquid chromatography/mass spectrometry (LC/MS/MS) for a comprehensive analysis of both nonpolar and polar pesticides and residual contaminants in food.

Robust pesticide analysis that supports a high-throughput workflow entails a series of requirements. It must provide extended maintenance-free operation with minimal downtime; it should meet the required sensitivity, which can be at sub-ppb levels; and it must enable calibration performance over a wide dynamic range that encompasses the MRLs for the compounds monitored.

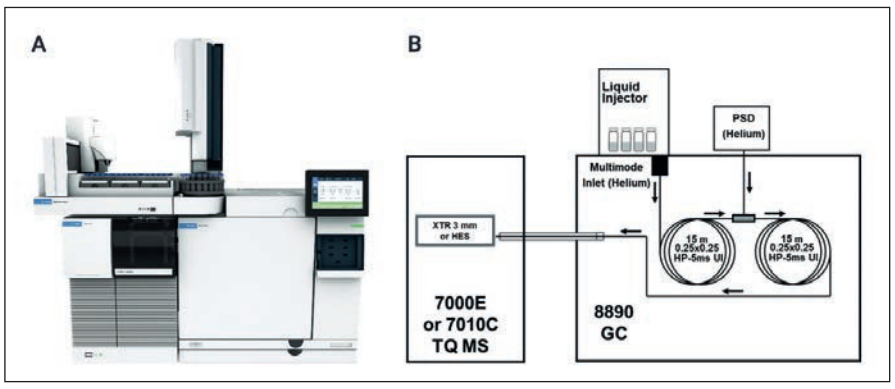

Figure 1. The Agilent 8890/7000E and 8890/7010 GC/QT system (A) and system configuration (B)

Our process enabled accurate quantification of pesticides in these matrices at their MRLs, achieving matrix-matched calibrations with R2 >0.99 over dynamic ranges as wide as 0.1 to 5,000 ppb with high method sensitivity. In addition, these strategies minimise instrument downtime, which is confined to liner and septum replacement about every 100 injections. Across 1,000 injections of the three complex matrix extracts (spinach, cayenne pepper, and walnut), there was no need to perform TQ MS tuning, source cleaning, or GC column trimming. The details on system performance during the longevity study can be found in this publication: https://www.agilent.com/cs/library/applications/an-gc-ms-ms-analysis-of-203-pesticides-in-10-minutes-in-spinach-5994-4967en-agilent.pdf

Sample Preparation

Effective sample extraction and matrix cleanup allow for minimal matrix background and interferences while maintaining high pesticide recoveries. Analysing crude QuEChERS extracts, especially of complex pigmented and oily matrices, can significantly increase the need for liner replacement, inlet cleaning, GC column trimming, and MS source

cleaning. Such maintenance procedures decrease throughput of the analysis. Performing an efficient matrix cleanup following QuEChERS extraction reduces in-source matrix loading and interferences with targets, while improving signal-to-noise ratio, accuracy, and reproducibility for target pesticides.

The sample preparation workflow, shown in *Figure 2* included two major steps:

Sample extraction by traditional QuEChERS extraction and an appropriate pass-through cleanup with Captiva EMR with Carbon S. These EMR with Carbon S cartridges can be adopted directly after QuEChERS extraction using the simplified pass-through procedure and demonstrates the improvement on both sample matrix removal and targets overall recovery and reproducibility. The Captiva EMR with Carbon S cartridges selection is based on the plant origin sample matrix complexity and pigmented level according to the selection guide from previous study. (https://www.agilent.com/cs/library/applications/an-captiva-emr-gpf-5994-4764en-agilent.pdf) *Figure 2* shows the entire flow chart for sample preparation.

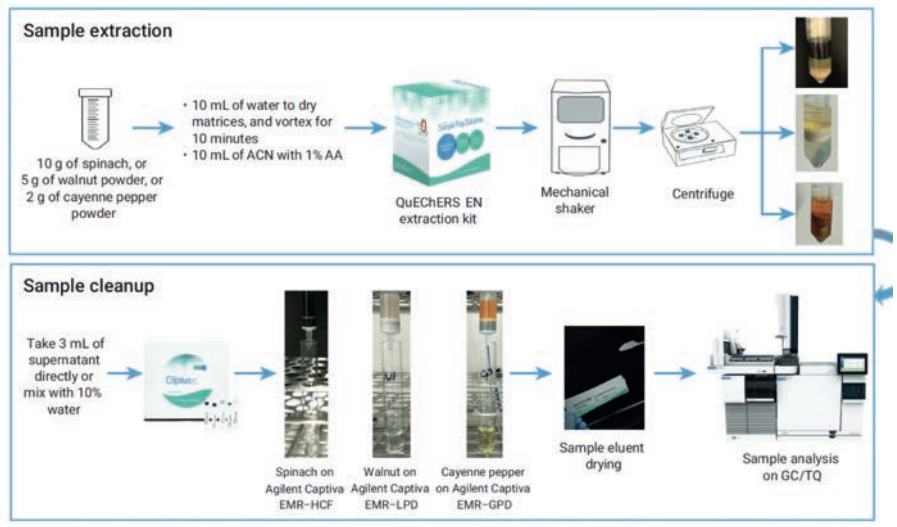

Figure 2. Sample preparation flowchart including traditional QuEChERS extraction, followed with Captiva EMR pass-through cleanup.

As shown in *Figure 3*, the abundance of TIC signal in full scan data acquisition mode was noticeably reduced for spinach, walnut, and cayenne pepper extracts after cleanup compared to crude extracts before cleanup.

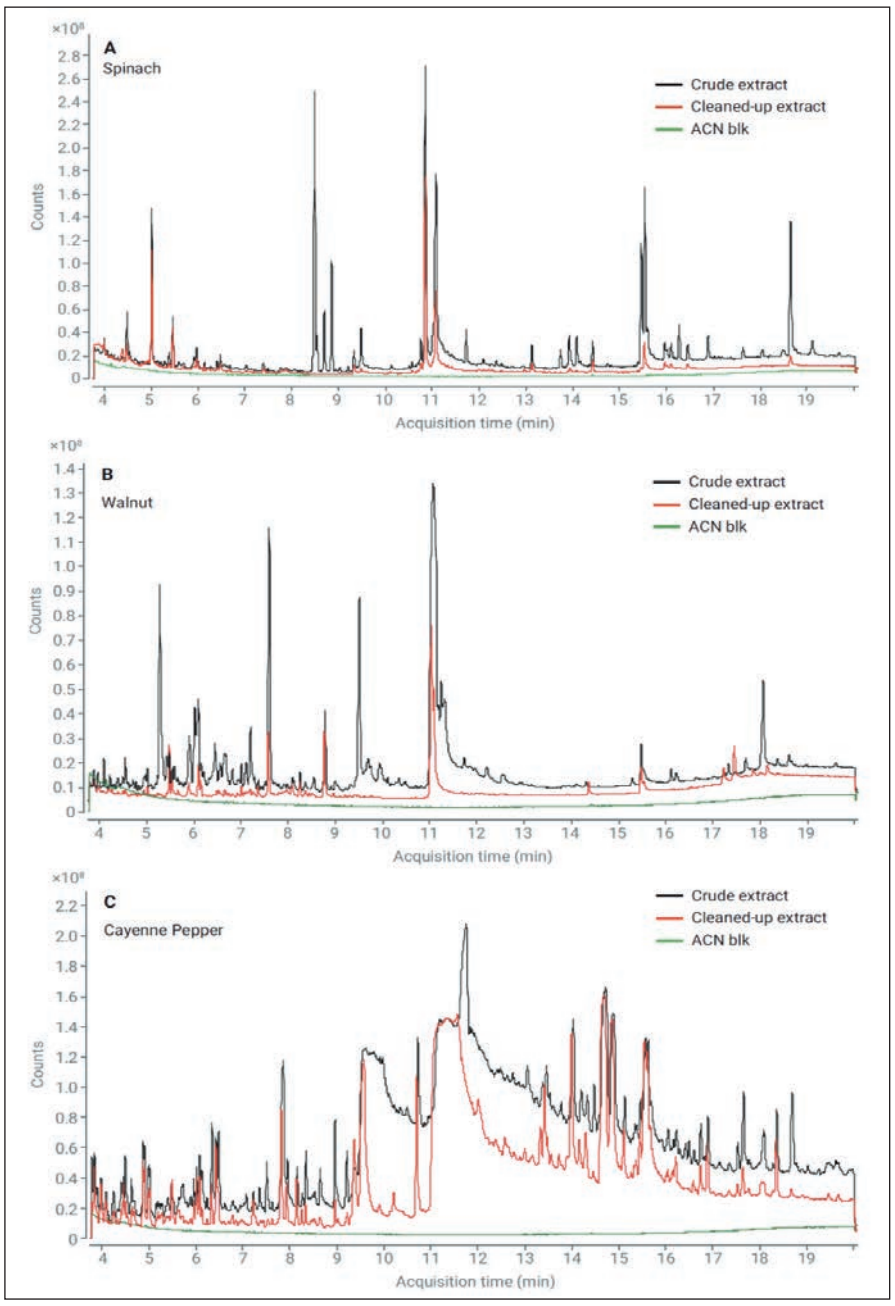

Figure 3. Scan TIC of the spinach (A), walnut (B), and cayenne pepper (C) extracts. The red trace corresponds to matrix sample with Captiva EMR cleanup and the black trace corresponds to matrix sample without clean up. The green trace corresponds to the acetonitrile solvent blank.

Matrix screening in full scan data acquisition mode

Screening samples in full scan data acquisition mode facilitates the evaluation of in-source matrix loading. To maintain optimal performance, every MS source limits the amount of material that can be present in the source at one time. If the electron ionisation (EI) source is overloaded with the matrix, quantitation accuracy of the analysis can be significantly compromised. Therefore, it is essential to analyse the matrix in full scan mode to evaluate total ion chromatogram (TIC) and maintain the optimal GC/TQ performance.

The abundance of TIC in full scan mode is recommended not to exceed 7 $\times 10^7$ counts when analysing with an electron multiplier (EM) gain set to 1. Out of the three analysed matrices, cayenne pepper featured the highest matrix background, although the background was noticeably reduced after the cleanup procedure. In this evaluation, pesticides that eluted between 11 and 12.5 minutes were expected to have sacrificed performance in the cayenne pepper matrix when evaluating sensitivity and the dynamic range. For example, Endosulfan I eluted at 11.273 minutes, and it could be quantitated only starting at 5 ppb in the cayenne pepper matrix with both GC/TQ systems. Spinach and walnut matrices had significantly lower matrix levels coeluting with Endosulfan I, with 0.1 ppb limit of quantification (LOQ) observed.

Some of the practices that can help lower the matrix background include adequate sample cleanup, sample dilution, and smaller injection volume. The latter two approaches often result in better LOQs, especially with the 7010C GC/TQ system equipped with a high efficiency source (HES).

Midcolumn backflushing

Midcolumn backflushing is a technique in which the carrier gas flow is reversed after the last analyte has exited the column. The use of the midcolumn backflushing configuration allows the analyst to limit the analysis time to the retention time of the last-eluting compound of interest. Challenging matrices – especially the oily ones, such as walnut - are rich in high-boiling components, with long retention times that exceed the retention times for the target pesticides. A common way to avoid ghost peaks in the subsequent runs was to use an extended column bake-out after the last target analyte eluted from the column.

However, this approach has several disadvantages, including the deposition of high boilers and GC column stationary phase into the EI source, contamination of the head of the GC column, a decrease of the column lifetime, and a longer cycle time due to the extended bake-out.

Midcolumn backflush allows the elution of the high-boiling matrix components from the column without the sacrifices encountered with the bake-out approach. After the MS data are collected, the oven is held at the final temperature in postrun mode, and the carrier gas flow through the first column is reversed. This reversed flow carries any high boilers that were in the column at the end of data collection. The high boilers are carried out of the head of the column and into the split vent trap (*Figure 4A*).

The ability to reverse the flow is provided by a tee that is inserted, in this case, between two identical 15 m columns. During the analysis, a small makeup flow of carrier gas is used to sweep the connection. During backflushing, the makeup flow is raised to a much higher value, sweeping high boilers backward out of the first column while simultaneously providing forward flow in the second column. For the configuration in this application, the backflushing time was 1.5 minutes.

The chromatograms shown in *Figure 4B* illustrate the effectiveness of the backflush technique in reducing cycle time sample carryover. The cycle time was reduced by 50%, and the columns did not have to be exposed to the higher bake-out temperatures for an extended time. Using backflush, excess column bleed and heavy residues are not introduced into the mass-spectrometer thereby reducing ion source contamination.

In addition, the midcolumn backflushing configuration provides a significant time-saving benefit when coupled with a multimode inlet. Maintenance procedures such as column trimming, and septum and liner change can be performed without the need to cool down the MS transfer line and source. When the septum is removed, the system's pneumatic switching device module provides the carrier gas flowing backward through column 1. It also prevents air from entering the GC columns and the MS. Multimode inlet fast cooling capability enables more time savings. As a result, liner and septum replacement, which are the most common maintenance procedures, can be performed in a few minutes.

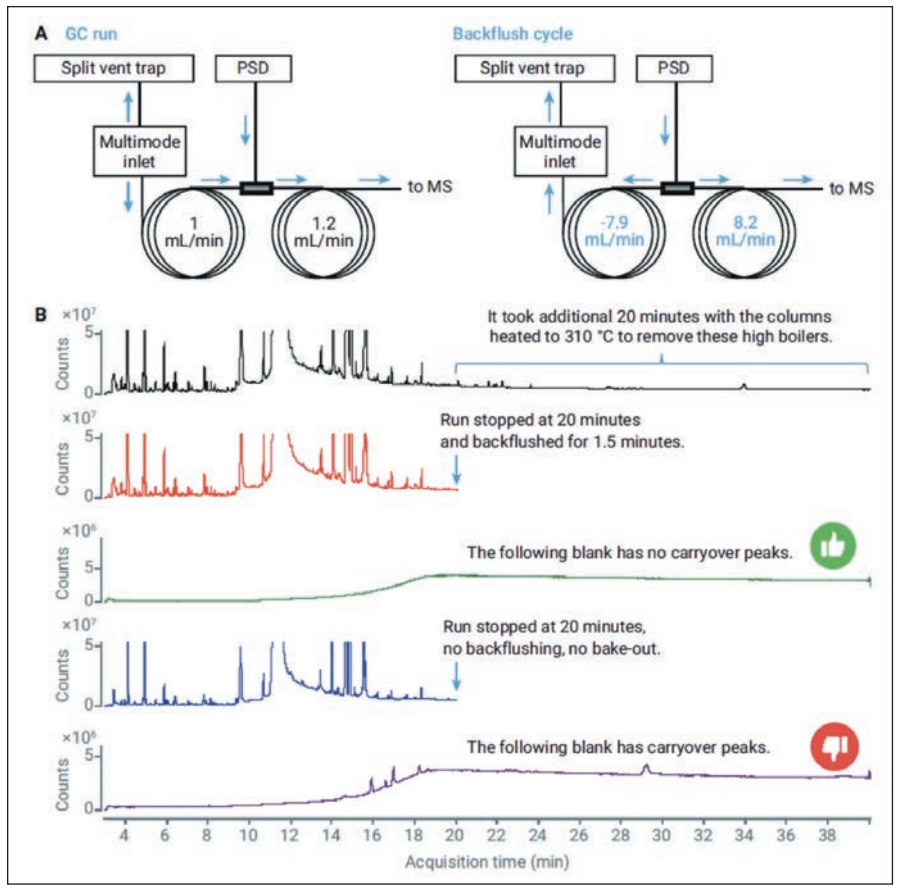

Figure 4. Midcolumn backflush configuration and gas flow during the GC run and the backflush cycle (A); TIC Scan chromatograms of a cayenne pepper extract followed by the analysis of an instrument blank with column bake-out, with backflush and without backflush or bake-out (B)

Leak-free GC/TQ system

Preventing leaks in a GC/MS system is essential for the long-term performance of the instrument. Undesired leaks reduce the GC column lifetime and lead to oxidation of the EI source, degrading its performance. Some tools that enable tight connections and make installation easy and reproducible include self-tightening collared column nuts for GC and gold-plated flexible metal ferrules.

The self-tightening collared column nuts have an innovative spring-driven piston. The piston continuously presses against the short graphite/polyimide ferrule, maintaining a leak-free seal even after hundreds of temperature cycles of the oven. The addition of the collar makes column installation into the GC inlet and MS transfer line easy and reduces the possibility of variation. The locking collar allows locking the column in place, for accurate and repeatable installation results, time after time.

When MS source maintenance is not required, the collared nut in combination with the column installation tool allows installation of the column into the MS without opening the side door. Gold-plated flexible metal ferrules are inert and provide exceptionally reliable sealing. They prevent formation of microleaks and help maintain high sensitivity of the GC/TQ.

The system's air/water check, or autotune report, can signal the presence of leaks. However, this approach does not help to identify the source of the leak. Additionally, it may miss microleaks like those that may be present at user connections.

Pentachloronitrobenzene presents a challenge for LC/MS analysis, so GC/MS analysis is the technique of choice.

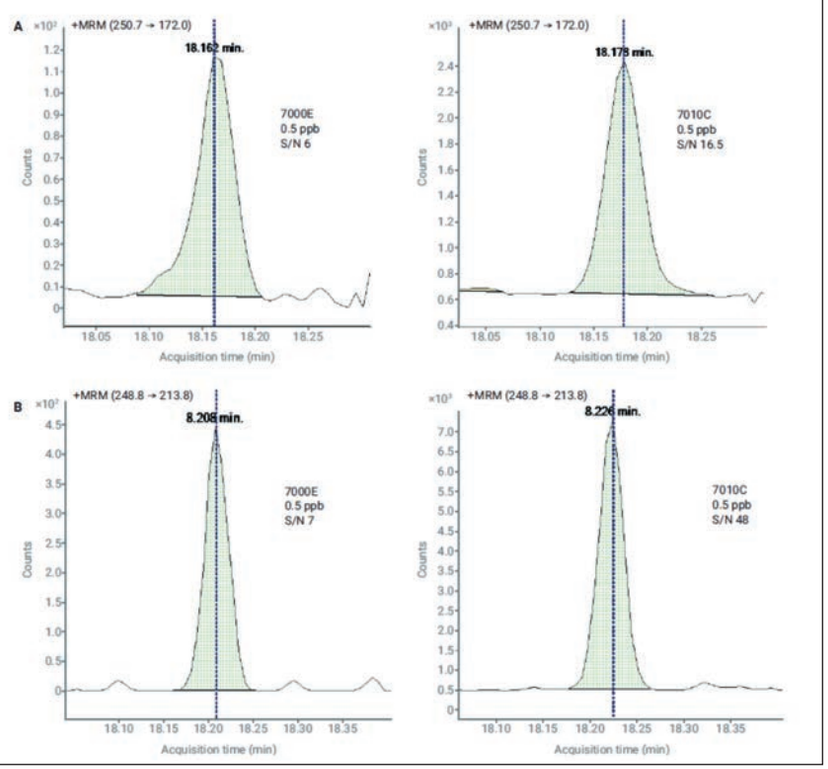

Figure 5. MRM chromatograms for deltamethrin (A) and pentachloronitrobenzene (B) at 0.5 ppb in walnut extract analysed with the 7000E and 7010 GC/TQ

With the recent advancements in GC/MS software, the source of the leak can be identified through monitoring user-specific ions and plotting corresponding chromatograms.

Optimised injection with the temperature-programmable multimode inlet

Efficiently volatilising the sample in the GC inlet is an essential component of a successful GC/MS analysis. Some pesticides, such as captafol, captan, dicofol, folpet, and deltamethrin, are known to suffer thermal degradation during injection. Starting the injection at lower temperature of 60°C and ramping up to 280°C allows for volatilising all the target analytes while maintaining their chemical integrity upon introduction to the GC column. Moreover, the ability to program the inlet temperature allows heating up the inlet further to 310°C during the post run while backflushing. This heating enables the system to bakeout any matrix residue that may remain in the inlet. The combination of temperature-programmable injection with a ultra-inert 2 mm dimpled liner resulted in high sensitivity, even for challenging pesticides like deltamethrin in a complex walnut matrix. *Figure 5A* shows the response of deltamethrin, a pesticide with an established MRL in walnuts, at 0.5 ppb.

Figure 5B shows the chromatograms for a selective multiple reaction monitoring transition for pentachloronitrobenzene, a commonly analysed pesticide, in a walnut extract. Pentachloronitrobenzene has established MRLs in many vegetables and fruits, peanuts, and soybean seeds that vary from 20 ppb to 1 ppm [4]. Pentachloronitrobenzene presents a challenge for LC/MS analysis, so GC/MS analysis is the technique of choice.

Multi-class, multi-residue pesticide GC/MS/MS testing allows for routine monitoring, enabling high throughput, sensitive detection levels, and rapid quantitative analysis for hundreds of pesticides in a single sample. Implementing these five key practices when analysing pesticides in challenging food matrices including spinach, walnut and cayenne pepper, allows for excellent calibration performance for the resulting method, over a wide dynamic range up to over four orders of magnitude. Consider implementing these measures to reduce matrix interferences with target analytes and extend your instrument's maintenance-free operation time.

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25 Year Anniversary: The Origin of Evaporating Cups

Elemental Microanalysis, a leading global supplier to the elemental analysis market, manufactures a product which continues to be useful in the market after 25 years.

The simple explanation of what the evaporating cup does, 'drying liquid samples down to residues prior to feeding into an elemental analyser', belies the amount of product engineering that went into designing a shape where the residue forms in the bottom of the cup while also allowing easy folding. That explanation also does not highlight the extremely stringent quality control measures which make Elemental's evaporating cups the leading choice among malting companies.

It started 25 years ago when Elemental was approached by a world-renowned Irish brewery which intended to specify a new, unique encapsulation product for drying their maltings sludge. They were working with an OEM on developing a robust, lightweight product with significant fluid capacity and dual laboratory use capability. The specific parameters of the encapsulation product included:

- 5ml liquid capacity
- Compatible with hotplate evaporator
- Easy to fold whilst containing dry residue
- Lightweight
- Suitable for combustion analysis

In collaboration with the brewery and the OEM, the design team at Elemental expended every effort to produce a tin foil evaporating cup that met the customer's exacting requirements. Elemental's team researched, designed, tested, developed, tested, further developed, checked, and tested again to product an appropriate product. Additionally, once the product was perfected, Elemental's team developed unique packaging to ensure safe product transit for the delicate evaporating cups.

Today, Elemental Microanalysis's ISO9001 accredited quality system continues to ensure that these evaporating cups - and all other analysis consumables - are of excellent quality at a competitive price. And malting companies across the globe have benefitted from the humble beginnings of the now indispensable evaporating cups.

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